

Appendix C

Prioritized List of Assumptions and Limitations of Data Used in the Risk Assessment

PRIORITY 1

ASSUMPTION: The fluoroquinolone resistance observed in persons ill from campylobacteriosis, (after removal of travelers, those who took a fluoroquinolone prior to culture and those for whom the time of taking the fluoroquinolone was unknown) is attributed to chickens. (Section 3.5)

DISCUSSION: It is difficult to know what proportion of the resistance in human campylobacteriosis may be attributable to a single source of human exposure when a level of resistance is defined in a population of cases whose exposures are multiple and varied. The level of resistance in the population may be consistent across all sources of human infection or may be disproportionately distributed with certain types of exposures carrying higher levels of fluoroquinolone resistance than other sources of human infection.

Fluoroquinolone use has been associated with the development of fluoroquinolone resistance in *Campylobacter* in clinical trials in poultry production units (41), in poultry production in the Netherlands (24) and in the United States (71) after the introduction of veterinary fluoroquinolones.

An Extra Label Use Prohibition of fluoroquinolone use in food-producing animals was published in 1997 (21CFR530.41), limiting food animal drug use to species listed on the product label. Approvals of fluoroquinolone drugs for use in animals include feline and canine oral and canine injectable products (available in 1989), poultry water soluble and in-ovo injectable products (available in 1995) and feedlot cattle injectable products (available in October, 1998). There are no fluoroquinolones currently approved for use in swine.

Campylobacteriosis is primarily an animal-derived foodborne disease, with the predominant source of human infections attributed to poultry (22, 31, 36 64). There is little surveillance data available to describe the level of fluoroquinolone resistance in *Campylobacter* isolated from animal derived food in the United States, either before or after the approval of these drugs for food animal use. Chicken *Campylobacter* isolates collected in 1998 indicated a level of 13% resistance to Ciprofloxacin (see Section 4.1). Because there was no food animal fluoroquinolone use other than use in poultry until late 1998, and no resistance was observed prior to 1992 in human cases¹ it is unlikely that the increase in domestically acquired fluoroquinolone resistance observed in people since 1996² can be attributed to a consistently distributed

¹ In two surveys encompassing 474 human isolates from 1982 to 1992 in the United States, only a single Ciprofloxacin resistant isolate was identified and subsequently speciated as *C. lari* (70).

² After removal of persons who had traveled within 7 days of illness onset and removal of those taking fluoroquinolones prior to culture, quinolone resistance in Minnesota was observed in 0.8% of isolates in 1996 and had increased to 3.0% in 1998 (chi square for linear trend, 9.8; $p \leq 0.002$) (71). In Minnesota quinolone resistance, screened by nalidixic acid disc diffusion was highly correlated with resistance to ciprofloxacin using the E-Test, (sensitivity 99.6%, specificity 98.4%) (71). A survey of *Campylobacter* isolated from 88% of 91 chicken products resulted in *C. jejuni* from 67(74%) and *C. coli* from 19 (21%) of samples and six samples were the source of both pathogens. Products carrying resistant isolates were purchased from 11 stores representing 8 franchises and originated in seven processing plants in five states (70, 71) indicating widespread resistance in chicken campylobacter isolates. Molecular subtyping was performed using PCR restriction endonuclease length polymorphism typing of the flagellin gene in the *C. jejuni* human and chicken product isolates. 12 subtypes were identified from 13 *C. jejuni* positive chicken products. Six of seven resistant subtypes in the chicken products were also identified in the quinolone resistant human isolates. For people acquiring infections during 1997, excluding cases that had taken fluoroquinolones prior to culture, persons with non-traveler resistant infections were more likely to have *C. jejuni* subtype also found in the quinolone resistant *C. jejuni* from chicken products (odds ratio 15.0, 95% CI 1.9 to 321.8) (70).

source of resistant *Campylobacter* exposures. Distribution of resistance from foodborne sources is more likely to be associated with specific exposures and limited predominantly to poultry.

DATA GAP: Quantification of the proportion of human disease attributable to various sources and the determination of the level of resistance carriage within the specific exposures would more precisely allow the determination of the relative contributions of the various exposures to fluoroquinolone resistant human disease. This ability to determine the relative contributions of various sources of infection to the level of resistance in human cases becomes increasingly important once fluoroquinolones are available for use in more than one food animal species. A model intended to determine the human health impact of the level of resistance in *Campylobacter* attributable to fluoroquinolone use in food animals will need to distribute the burden of resistant human disease among many different food animal species and attribute levels of resistance to sources of human infection. The level of resistance was not adjusted to account for the uneven distribution of resistance in sources of campylobacteriosis because the data were not sufficiently robust and presented a problem in the logic used in the model.

PRIORITY 2

ASSUMPTION: The level of risk ascertained in studies in the 1980's represents the current level of risk. (Section 3.1)

LIMITATIONS of studies used to determine the proportion of chicken associated cases:

Limitations of study 1 include: the demographic characteristics of the population, the frequency of chicken consumption, the proportion of the population consuming chicken and many other factors may have changed since this study. For example, the amount of chicken consumed has increased since 1982, and in 1998 people consumed 54.4% (72.60/47.02) more chicken, calculated in RTC pounds consumed per capita (80).

Limitations of study 2 include the lack of representativeness of the study population and the absence of some exposures, such as travel and raw milk that are frequently associated with risk in the population at large. In addition, the study was limited to enteric illnesses because more invasive infections were not eligible for inclusion in the study, although these usually comprise less than 1% of cases. These differences result in difficulty in generalizing the findings to the United States population but may represent the level of risk in some subgroups of the population.

Limitations of study 3 are similar to study 1.

DISCUSSION: In the three case control studies two indicated an increased risk of infection associated with consumption of chicken, and all three studies indicated an increased risk of campylobacteriosis associated with consumption of undercooked chicken. Two studies also indicated a risk associated with raw milk consumption although the proportion of attributable risk is much less than that attributed to chicken. A similar proportion of disease was attributable to chicken consumption in Studies 1 and 3, approximately 48%. The high estimate of attributable risk in the university student population indicates that in some subgroups of the population exposures are likely to differ and risk attributable to chicken will vary accordingly. These estimates of the etiologic fraction represent a range of risk that is likely to reflect the degree of risk in the early 1980's. More recent data do not exist for United States populations. Data analysis of a Case Control Study, conducted by the CDC and participating State Health Departments (CA, CT, GA, MD, MN, NY, OR), in 1998 within FoodNet sites is currently underway and will be published in the near future. The data from this study will provide updated risk factor information from which etiologic fractions associated with identified risk factors may be determined.

PRIORITY 3

ASSUMPTION: If a carcass is positive for *Campylobacter*, the predominant species isolated is *C. jejuni*.

LIMITATIONS of the prevalence estimate for ciprofloxacin resistance in *Campylobacter* from chickens includes: the small number of isolates collected, the lack of seasonal representation and potential for selection of mixed colonies of organisms when selecting a single colony. Many varied *Campylobacter* colonies are present on a culture plate. The selection of a single colony from a plate of diverse colonies provides a “plate average” or is equivalent to the proportion of resistant isolates on a carcass, rather than a carcass prevalence, assuming that isolates on carcasses reflect culturable isolates. Therefore, the 11.3% estimate of the level of resistance may under-represent the carcass prevalence of resistant *Campylobacter* isolates. Use of a quinolone-containing screening media would provide a better estimate of the carcass prevalence of Ciprofloxacin resistant *Campylobacter*. (Section 4.2)

Hippurate positive isolates were tested in one laboratory using *Campylobacter* species specific PCR primers for the *ceu* gene and preliminary findings indicated that approximately 8.2% of hippurate positive animal isolates reacted with *C. coli* specific primers (personal communication P. Fedorka-Cray 10/1/99). This potential bias has not been identified in another laboratory and since the technique is very sensitive and has only been identified in a single laboratory, the information was not considered relevant to the risk assessment.

DATA GAP: A yearlong survey of broiler chicken carcasses to allow the estimation of a yearly prevalence of *Campylobacter* and the testing of those isolates from broilers for Ciprofloxacin resistance is currently underway with FSIS. These isolates will be tested for susceptibility using the E-Test and data for 1999 should be available in mid-2000.

PRIORITY 4

ASSUMPTION(s): The rate of seeking care among persons with bloody stools is similar to the rate of seeking care among persons with campylobacteriosis with bloody stools. The rate of seeking care for diarrheal illness among persons with non-bloody stools is similar to the rate of seeking care among persons with campylobacteriosis with non-bloody stools. (Section 1)

DISCUSSION: These estimates were for all diarrheal disease, and not specific to campylobacteriosis. A recently published rate for seeking care for campylobacteriosis in the U.S. was not available from the literature or other sources. Bacterial foodborne disease is typically more severe than viral foodborne disease (28, 96) and rates of seeking care are likely to differ by pathogen.

In the population survey factors that were most important in influencing the decision to seek care were identified as fever, vomiting, “how sick they felt,” stomach cramps, blood in stool and duration of diarrhea (1). Some of these factors were evaluated for all diarrheal disease in the telephone survey and compared with the same characteristics in individuals who had culture-confirmed *Campylobacter* infections or diarrheal disease (Table 3). Comparing the groups, a greater proportion of persons with culture-confirmed *Campylobacter* cases were affected by reported fever and blood in the stool than the population of persons seeking care for all diarrheal disease. Therefore, the actual rate of seeking care for campylobacteriosis may be somewhat underestimated by the overall 12% rate estimate (12% non-bloody and 15% bloody stools). However, because a greater proportion of persons with fever and bloody stools are likely to be cultured and enrolled in the case control study, such comparisons are difficult.

DATA GAP: Additional studies to define the rate of seeking care for campylobacteriosis would be helpful and would provide a more accurate estimate of disease incidence and rate of seeking care but would require very large community based surveys that are likely to require considerable resources to conduct. Another alternative suggested by CDC is to develop symptom multipliers for diseases that could be used to determine care seeking rates.

PRIORITY 5

The following four assumptions are made because *Campylobacter* specific data are not available and rank similarly in magnitude in terms of being a limitation of the model.

ASSUMPTION: The incidence rates for culture-confirmed *Campylobacter* infections in the FoodNet catchment are representative of incidence rates for culture-confirmed *Campylobacter* infections in the United States. (Section 1.4)

DISCUSSION: Although the 1998 incidence rates varied by site, from 10.2/100,000 in Maryland to 37.7/100,000 in California in a preliminary report (18), the overall rate of *Campylobacter* isolation is likely to reflect isolation rates in the U.S. population because comparisons of demographic characteristics between the FoodNet sites and the U.S. population show similar distributions of sex, age, race and rural/urban distributions. Furthermore, the frequency of chicken consumption indicated very minor differences (see section 1.2 for exposure to chicken) when compared by similar demographic characteristics.

ASSUMPTION: The probability that a stool specimen was requested among persons with bloody stools is similar to the probability that a stool specimen was requested among persons with campylobacteriosis with bloody stools. The probability that a stool specimen was requested among persons with non-bloody stools is similar to the probability that a stool specimen was requested among persons with campylobacteriosis with non-bloody stools. (Section 2.2)

ASSUMPTION: Over-reporting by physicians of the proportion of persons with bloody diarrhea that are requested to submit stool specimens, compared to the proportion of stool requests reported from the persons with bloody diarrhea, is similar to physician over-reporting for persons without observable blood in their stools. (Section 2.2)

DISCUSSION: There is little information on the sensitivity of stool culture methods and the methods for culturing stools are extremely diverse. Specimen handling is another factor that can greatly decrease the sensitivity of stool culture methods. In a review of non-typhoidal salmonellosis, an assumed estimate of the sensitivity of culture was 70% and was used to estimate the burden of salmonellosis in the United States (2). This estimate was adopted for determining the burden of campylobacteriosis in a recent review of foodborne disease (9).

DATA GAP: Incomplete knowledge of the sensitivity and specificity of culturing specimens for *Campylobacter* exists.

ASSUMPTION: The population survey proportion of cases of all acute diarrheal illness seeking care, not submitting a stool sample and receiving an antibiotic (40%) is similar to that for persons ill with campylobacteriosis. (Section 3.3)

PRIORITY 6

The following assumptions all relate to invasive disease and since invasive disease represents less than 1% of all cases these assumptions will have minimal impact on the model.

ASSUMPTION: All invasive campylobacteriosis cases seek care, have a specimen collected which yields *Campylobacter* and is ascertained by FoodNet. (Section 1.3)

DISCUSSION: It is not known precisely what proportion of persons with invasive *Campylobacter* infections seek care, but because persons with invasive *Campylobacter* infections will be moderately to severely ill, it is likely that most of these patients will seek care.

There is little knowledge of the completeness of ascertainment of invasive campylobacteriosis; the frequency with which laboratories are requested to test blood, CSF or other sterile specimens for *Campylobacter* and the sensitivity and specificity of the diagnostic tests used for isolation from blood and other sterile sites. Blood cultures usually represent more than 99% of all invasive isolations and most currently used blood culture systems are good for isolating *Campylobacter*, when it is present. The lack of information on the frequency of diagnostic requests and sensitivity may result in an underestimate of actual invasive disease rates. However, because the currently ascertained proportion of invasive cases is very small, approximately 1.0% of all confirmed cases, and most cases are likely to seek care, an increase in isolation of specimens classified as invasive is unlikely to have much impact on the overall number of cases of campylobacteriosis in the U.S.

DATA GAP: Data describing rates or cases of invasive disease seeking care, requests for diagnostic tests and the sensitivity of diagnostic procedures, such as blood culture, are not available.

ASSUMPTION: Because of the severity of illness upon presentation, all cases with invasive disease are presumed to take antibiotics for their illness. (Section 3.4)

DISCUSSION: Persons ill with campylobacteriosis, in general, present with more severe gastrointestinal symptoms to health care providers than *Salmonella*, *Shigella* or a comparison group of patients with diarrhea in a multi-center survey (14). Severity of illness is one of many factors that lead physicians to prescribe antibiotics to patients with a diarrheal illness.

ASSUMPTION: Patients with invasive campylobacteriosis were treated by their health care provider with fluoroquinolones at the same relative frequency as those patients with enteric disease who submitted stools for culture. (Section 3.4)

ASSUMPTION: The population survey proportion of cases of all acute diarrheal illness seeking care, not submitting a stool sample and receiving an antibiotic is similar to that for persons ill with campylobacteriosis. (Section 3.4)